

LISTING OF ALL CLAIMS AMENDED RELATIVE TO ORIGINAL PATENT
(CUMUMLATIVE TO DATE)

1. (amended) A monoclonal antibody specific for a purified human colon carcinoma-associated protein antigen, [wherein said antigen has the following characteristics:
(a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;
(b) said antigen is not detectable on normal colon cancer free human tissues;
(c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;
(d) said antigen is specifically immunogenic in humans; and
(e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity] which is murine monoclonal antibody 33.28 as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413.
2. (amended) [An] A purified antibody [according to claim 1 which is mouse monoclonal antibody 33.28 (ATCC HB-12315) or an antibody which binds specifically to a colon carcinoma-associated epitope that specifically binds to monoclonal antibody 3328] which competitively inhibits binding of the antibody of claim 1 to the human colon carcinoma - associated protein antigen.
3. (amended) An antibody according to claim 2 wherein said colon carcinoma-associated antigen is a protein having a molecular weight of about 61.1 kilodaltons as measured by gradient polyacrylamide gel electrophoresis.
4. (amended) [An antibody according claim 1] A monoclonal antibody specific for a purified human colon carcinoma-associated protein antigen, which is mouse monoclonal antibody 31.1, as produced by hybridoma PCA 31.1 [(ATCC HB-12314)] , deposited with the American Type Culture Collection and assigned accession number PTA-2497[

or an antibody which binds specifically to a colon carcinoma-associated epitope that specifically binds to monoclonal antibody 31.1].

5. (amended) [An] A purified antibody [according to claim 4 wherein said colon carcinoma-associated antigen is a protein having a molecular weight of about 72 kilodaltons] which competitively inhibits binding of the antibody of claim 4 to the human colon carcinoma - associated protein antigen.

6. (amended) An antibody according to claim [2] 5 wherein said colon carcinoma-associated antigen is a glycoprotein, the protein component having a molecular weight of [61.1] about 72 kilodaltons kilodaltons as measured by gradient polyacrylamide gel electrophoresis.

7. (amended) An antibody according to claim 1, 2, 4 or 5 immobilized on a solid phase.

8. (amended) An antibody according to claim 1, 2, 4 or 5 which is detectably labeled.

10. (amended) An antibody according to claim 1, 2, 4 or 5 conjugated to a cytotoxic radionuclide.

11. (amended) An antibody according to claim 1, 2, 4 or 5 conjugated to a cytotoxic drug.

12. (amended) An antibody according to claim 1, 2, 4 or 5 conjugated to a cytotoxic protein.

[16. A monoclonal antibody against the monoclonal antibody of claim 1.]

[17. A monoclonal antibody against the monoclonal antibody of claim 2.]

[18. A monoclonal antibody against the monoclonal antibody of claim 3.]

[19. A monoclonal antibody against the monoclonal antibody of claim 4.]

[20. A monoclonal antibody against the monoclonal antibody of claim 5.]

[21. A monoclonal antibody against the monoclonal antibody of claim 6.]

22. (amended) An immunoassay for detecting a colon carcinoma-associated antigen which binds to mouse monoclonal antibody 33.28 [(ATCC HB-12315)] as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413, in a sample comprising:

(a) contacting said sample with an effective binding amount of the antibody according to claim 1 or claim 2; and

(b) detecting said antigen by detecting the binding of the antibody to the [purified] colon carcinoma - associated protein antigen.

23. (amended) An immunoassay for detecting a colon carcinoma-associated antigen which binds to mouse monoclonal antibody 31.1 [(ATCC HB-12314)] , as produced by hybridoma PCA 31.1, deposited with the American Type Culture Collection and assigned accession number PTA-2497, in a sample comprising:

(a) contacting said sample with an effective binding amount of the antibody according to claim [1] 4 or claim 5; and

(b) detecting said antigen by detecting the binding of the antibody to the [purified] colon carcinoma - associated protein antigen.

24. (amended) A method for diagnosing colon cancer in humans comprising:

(a) removing a histological specimen from a patient suspected of having a colon cancer;

(b) contacting the specimen with monoclonal antibody 33.28 [(ATCC HB-12315)] , as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413;

- (c) staining the specimen with an immunohistochemical stain; and
- (d) detecting the presence of the antigen-antibody complex by the stain.

25. (amended) A method for diagnosing colon cancer in humans comprising:

- (a) removing a histological specimen from a patient suspected of having colon[-]carcinoma;
- (b) contacting the specimen with mouse monoclonal antibody 31.1 [(ATCC HB-12314)] , as produced by hybridoma PCA 31.1, deposited with the American Type Culture Collection and assigned accession number PTA-2497;
- (c) staining the specimen with an immunohistochemical stain; and
- (d) detecting the presence of the antigen-antibody complex.

28. (amended) A kit for the immunohistochemical detection of colon carcinoma comprising:

- (a) mouse monoclonal antibody 31.1[(ATCC HB-12314)] , as produced by hybridoma PCA 31.1, deposited with the American Type Culture Collection and assigned accession number PTA-2497;
- (b) reagents for immunoperoxidase and secondary antibody;
- (c) immunoperoxidase; and
- (d) colorizing reagents.

29. (amended) A kit for the immunohistochemical detection of colon carcinoma comprising:

- (a) mouse monoclonal antibody 33.28 [(ATCC HB-12315)] , as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413;
- (b) reagents for immunoperoxidase and secondary antibody;
- (c) immunoperoxidase; and
- (d) colorizing reagents.

30. (amended) A compartmentalized kit for the detection of a human colon carcinoma-associated antigen, [wherein the antigen has the following characteristics:

(a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;

(b) said antigen is not detectable on normal colon cancer free human tissues;

(c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;

(d) said antigen is specifically immunogenic in humans; and

(e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity,]

said kit comprising a first container adapted to contain an antibody according to claim 2 or 5[to said antigen or an active component thereof], and a second container adapted to contain a second antibody to said antigen [or an active component thereof], said second antibody being labeled with a reporter molecule capable of giving a detectable signal.

33. (amended) A kit according to claim [30] 32 wherein the kit further comprises a third container adapted to contain a substrate for the enzyme.

34. (amended) A compartmentalized kit for the detection of a human colon carcinoma-associated antigen, [wherein the antigen has the following characteristics:

(a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;

(b) said antigen is not detectable on normal colon cancer free human tissues;

(c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;

(d) said antigen is specifically immunogenic in humans; and

(e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity,]

said kit comprising a first container adapted to contain monoclonal antibody 31.1 [(ATCC HB-12314)], as produced by hybridoma PCA 31.1, deposited with the

American Type Culture Collection and assigned accession number PTA-2497, to said antigen and a second container adapted to contain a second antibody to said antigen [or an active component thereof], said second antibody being labeled with a reporter molecule capable of giving a detectable signal.

36. (amended) A kit according to claim [32] 34 wherein the reporter molecule is an enzyme.

37. (amended) A kit according to claim [33] 36 wherein the kit further comprises a third container adapted to contain a substrate for the enzyme.

38. (amended) A compartmentalized kit for the detection of a human colon carcinoma-associated antigen, [wherein the antigen has the following characteristics:

(a) said antigen is purified to the extent that the membrane fractions are free of HL-A antigen and are substantially free from non-immunogenic glycoprotein fractions;

(b) said antigen is not detectable on normal colon cancer free human tissues;

(c) said antigen is not detectable on human carcinoma cells other than colon carcinoma cells;

(d) said antigen is specifically immunogenic in humans; and

(e) said antigen induces an immune response in humans having colon carcinoma which is expressed as cell mediated immunity,]

said kit comprising a first container adapted to contain monoclonal antibody 33.28, as

produced by hybridoma PCA 33.28, deposited with the American Type Culture

Collection and assigned accession number PTA-5413 [(ATCC HB-12315)] to said

antigen and a second container adapted to contain a second antibody to said antigen [or an active component thereof], said second antibody being labeled with a reporter molecule capable of giving a detectable signal.

41. (amended) A kit according to claim [38] 40 wherein the kit further comprises a third container adapted to contain a substrate for the enzyme.

[42. The monoclonal antibody of claim 1 which is a chimeric antibody.]

43. (amended) [The] A chimeric antibody [according to claim 42] which is a chimeric mouse/human antibody Chi #1 as produced by the cell line deposited with the American Type Culture Collection and assigned accession number [(ATCC) CRL-12316[]].

44. (amended) The chimeric antibody according to claim [42] 43 wherein said colon carcinoma-associated antigen is a protein having a molecular weight of 72 [kilodalton] kilodaltons as measured by gradient polyacrylamide gel electrophoresis.

45. (amended) A composition comprising the chimeric antibody according to claim [42] 43 in combination with a pharmaceutically acceptable carrier.

[46. A monoclonal antibody against the chimeric antibody of claim 42.]

47. (amended) An immunoassay for detecting a colon carcinoma-associated antigen which binds to the mouse/human chimeric antibody Chi #1 as produced by the cell line deposited with the American Type Culture Collection and assigned accession number [(ATCC) CRL-12316[]] of claim 42] in a sample comprising:

- (a) contacting said sample with the Chi #1 antibody [according to claim 42]; and
- (b) detecting said antigen by detecting the binding of said antibody to the [purified] colon carcinoma - associated protein antigen.

48. (amended) A method for diagnosing colon cancer in humans comprising:

- (a) removing a histological specimen from a patient suspected of having a colon carcinoma;
- (b) contacting the specimen with a chimeric antibody [which binds to an antigen] according to claim [1] 43;
- (c) staining the specimen with an immunohistochemical stain; and

(d) detecting the presence of the antigen-antibody complex by the stain.

49. (amended) A method for diagnosing colon cancer in humans comprising:

(a) removing a histological specimen from a patient suspected of having a colon carcinoma;

(b) contacting the specimen with mouse/human chimeric antibody which binds to an antigen which binds to mouse/human chimeric antibody Chi #1 [(ATCC) as produced by the cell line deposited with the American Type Culture Collection and assigned accession number CRL-12316()];

(c) staining the specimen with an immunohistochemical stain; and

(d) detecting the presence of the antigen-antibody complex by the stain.

51. (new) A purified antibody which is raised against an immunopurified human colon carcinoma associated antigen that is specifically bound by monoclonal antibody 31.1 , as produced by hybridoma PCA 31.1, deposited with the American Type Culture Collection and assigned accession number PTA-2497.

52.(new) The antibody of claim 51 which is a monoclonal antibody.

53. (new) An antibody which is raised against a purified human colon carcinoma associated antigen that is specifically bound by monoclonal antibody 33.28, as produced by hybridoma PCA 33.28, deposited with the American Type Culture Collection and assigned accession number PTA-5413.

54. (new) The antibody of claim 53 which is a monoclonal antibody.